MNCs for 7d in RPMI-1640 + 10 IU IL-2. NKR expression (KIR2DS4, NKG2D, NKG2A, CD94, KIR3DL1, KIR3DL2, Nkp46), LAMP-1 receptor expression and NK cell phenotype ($CD56^{dim/bright}$ subsets) determined by flow cytometry. On Day 0, $CD3^-/56^+$ NK cell subset was 3.0 ± 1.3%. After 7d culture, NK cells increased to 71.7 ± 3.9%, compared to media alone (9.7 ± 2.4%) and WTK562 (42.6 ± 5.9%,$p < 0.01$). This represented a 35-fold or 3374 ± 385% increase of input NK cell number. CB Tcells were decreased compared to media alone and WTK562 (15.1 ± 1.7 vs 51 ± 7.1 vs 35.7 ± 2.4%,$p < 0.001$). $CD56^{bright}$ vs $CD56^{dim}$ subsets were increased (67 vs 33%,$p < 0.01$) following K562-mbIL15-41BBL stimulation. CB NK cells expressing KIR3DL1 were increased 10-fold following stimulation with K562-mbIL15-41BBL vs WTK562 ($p < 0.01$) and 5-fold increase in NK KIR2DS4 expression ($p < 0.05$), respectively. NK activation marker, CD107a was increased compared to WTK562 (51 ± 0.7 vs 32 ± 1.1%, $p < 0.05$). Since a standard cryopreserved UCB unit contains approximately 75×10^7 MNCs, by using the smaller aliquot (5 ml or 20%) 150×10^6 MNCs x 3.9% = 5.8×10^6NK cells, this method may yield 2.0×10^8 CB NK cells after 7d culture. This suggests that CBMNC can be expanded by K562-mbIL15-41BBL resulting in increased NK cell KIR expression (KIR2DS4, KIR3DL1) and NK activation (CD107a) with a decrease in T cells which may provide a means to enhance specific CB NK expansion for ACI in post UCBT setting.

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VIABILITY AND POTENCY OF HEMATOPOIETIC PROGENITOR CELLS AFTER PROLONGED CRYOPRESERVATION AT -80 °C

Khattab, M., Gilmore, G.L., Sabovic, E.A., Miller, S.M., Rossetti, J.M., Abdulhaq, H., Lister, J. The Western Pennsylvania Cancer Institute, Pittsburgh, PA

Hematopoietic progenitor cell (HPC) products cryopreserved in the liquid or vapor phase of liquid nitrogen (LN) remain viable and potent for years. Cryopreserved HPC products when stored at -80 °C in mechanical freezers are known to remain viable and potent for up to 180 days. We demonstrated viability and potency of HPC products stored at -80 °C for up to 12 years. Products were cryopreserved in 6% hydroxyethyl starch, 5% DMSO and 4% human serum albumin using non-controlled rate freezing.

Thawed products were tested for viability and potency respectively by trypan blue dye exclusion and hematopoietic progenitor cell colony assay (CFU-GM, BFU-E, CFU-GEMM). All culture assays used MethoCult® GF^+ H4445 (STEMCELL Technologies, Vancouver, BC, Canada).

We thawed 20 HPC products collected from mobilized peripheral blood. The products were stored between 7 and 12 years at -80 °C. In 19 cases, trypan blue dye exclusion yielded an average 72% viability (range: 50%-89%). HPC colony assay yielded average progenitor frequencies of: CFU-GM 159 [range: 15-600]; BFU-E 116 [range: 2-388] and CFU-GEMM 21 [range: 2-68] all per 10^5 viable cells plated. The average values did not differ from control values of CFU-GM 111, BFU-E 121 and CFU-GEMM 23. Two products were infused into their donors after myeloablative conditioning, UPN 947 and UPN 1000. The products were stored for over 9 years. The products from UPN 947/UPN 1000 gave 73%/65% viability by trypan blue dye exclusion, CFU-GM 347/508, BFU-E 135/344, CFU-GEMM 12/48 per 10^5 viable cells, ANC500 day + 14/day + 11 and PLT50 day + 15/day + 24.

We conclude that viability and potency of these HPC products flash-frozen and stored at -80 °C in pentastarch is maintained for up to 12 years. In two cases engraftment was achieved with long-term cryopreserved products. The data suggest that prolonged cryopreservation of HPC products using LN may not be necessary to preserve product potency.

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DEVELOPMENT OF THE "MARROWMINER": A NOVEL, MINIMALLY INVASIVE DEVICE OF FOR THE HARVEST OF BONE MARROW. FROM BENCH-TOP, TO ANIMAL STUDIES, THROUGH FDA APPROVAL AND HUMAN EVALUATION

Kraft, D.[1], Crocker, M.[2], Ghazarossian, V.[2], Antonio, C.A.[3] [1] Stanford University, Stanford, CA; [2] StemCor Systems Inc, Menlo Park, CA; [3] Sangre De Cordon SA, Guadalajara, Mexico

Bone marrow (BM) is the traditional graft source in hematopoietic stem cell transplantation. An increasing body of work suggests that use of BM grafts may show long-term advantages over mobilized PBSC in some alloegeneic transplant settings, resulting in significantly less cGVHD, morbidity and improved survival. Traditional OR based BM harvest methods however remain crude, labor and resource intensive, generally requiring full general anesthesia, 2 transplant clinicians, 100+ serial small volume needle aspirates, and result in grafts highly diluted by peripheral blood. Improved harvest methods are needed.

To address the need to develop improved methods for harvest, The "MarrowMiner" (MM) was conceived. The MarrowMiner (MM), is a novel, now FDA cleared & CE-Marked device developed for the minimally invasive harvest of BM to enable the rapid, convenient, outpatient harvest of large quantities of BM via a single marrow entry site, under local anesthesia. A powered, rotating FlexShaft enables the clinician to access a broad area of the ileac marrow space through a single cortical bone entry site trocar. The FlexShaft moves through the cancellous bone, without puncturing the cortical wall, while aspirating rich marrow under negative pressure into a closed container.

Preclinical trails in porcine models compared the marrow harvested with the MM to standard 6-hole harvest in the opposite ileac. This 6 pig study revealed a significantly richer bone marrow with >9 fold Colony Forming (CFU-F) per ml compared to standard harvest. Following FDA approval, successful 'First in Man' studies were conducted demonstrating safety and efficacy in patients having marrow harvested for regenerative medicine studies.

A 21 patient trial comparing the MM to standard harvest. The MARVELOUS (MARrowMiner VErsus standard ILeac bOne marrow pUncture and aSpiration)) study found that MM aspirate (performed under local anesthesia) had a greater average TNC count/ml compared to standard marrow harvest in the same patient, with equivalent viability. Higher numbers of %ALDH+, CD34+, phenotypic MSC) were obtained by MM.

The novel MM system demonstrated safety and efficacy in clinical use. An ongoing trial is evaluating the MM in the BMT setting, and a commercial version of the device is nearing production. The MM device device may benefit ease of harvest for donors, clinicians and could ultimately lead to better outcomes for transplant recipients.

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DETECTION AND EX VIVO EXPANSION OF DORMANT ANTI-VIRAL CTL PRECURSORS ISOLATED FROM RECIPIENTS OF UNRELATED UMBILICAL CORD BLOOD TRANSPLANT (UCBT)

Marti, L.C.[1], Leen, A.M.[2], Janetzki, S.[3], Baker, J.H.[1], Szabolcs, P.[4] [1] Duke University Medical Center, Durham, NC; [2] Baylor College of Medicine, Houston, TX; [3] Zellnet Consulting, Fort Lee, NJ; [4] Duke University Medical Center

Viral infections are commonly detected within weeks after UCBT that could trigger *in vivo* priming of the infused naïve T cells. This hypothesis is supported by the observation that infections can resolve without antiviral therapy.

Objectives: In this study we focused on two potentially fatal viruses, CMV and adenovirus to assess the acquisition and evolution of antigen-specific immunity. We hypothesized that 1) threshold numbers of CTL precursors can be identified for each virus that may influence outcome 2) CTL precursors may reside below the level of detection during GVHD prophylaxis, however, ex vivo restimulation could expand them to measurable frequencies.

Methods: Monocytes infected with an adenovirus vector carrying the CMV pp65 transgene (Ad5f35pp65) were used as APC in the presence of IL7. Antiviral CTLp was quantitated by ELISPOT with computerized enumeration of IFNg producing spot forming cells (SFC) in response to peptide pools spanning dominant viral antigens: hexon and penton for adenovirus, pp65, EI-1 for CMV.

Results: 8 infected patients at a median age of 9.9 years have been studied. Blood was drawn at a median of 70 days (range 34-470). Only 1 of 3 patients with adenovirus infection (stool x1, urine x1, respiratory tract x 1) had associated viremia while all five (5) patients with CMV had viremia. Analyzing freshly drawn peripheral blood 1 of